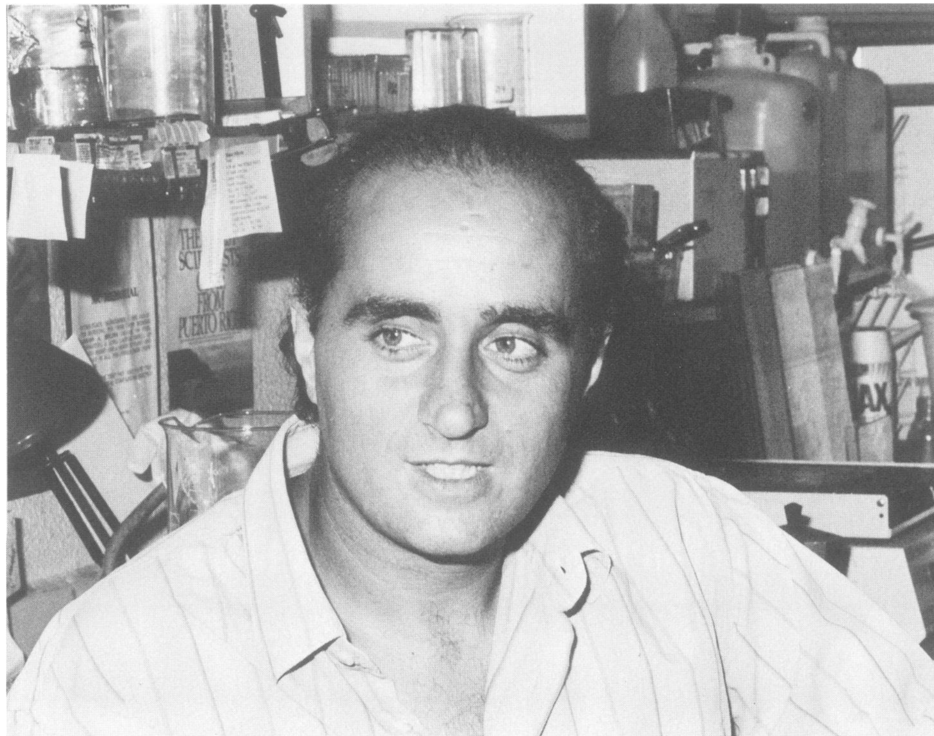

The EMBO Medal for 1994 has been awarded to
Dr Paolo Sassone-Corsi of the Laboratoire de Génétique Moléculaire des Eucaryotes, CNRS, Strasbourg, France, for his work on signal transduction and gene regulation, about which he writes in the following review.

The EMBO Journal vol.13 no.20 pp.4717–4728, 1994

MEDAL REVIEW

Goals for signal transduction pathways: linking up with transcriptional regulation

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From the beginning

As a young kid in Naples, Italy, my major concerns were football and science. While my real dream at that time was to became a great football player, I am not too upset today about being a scientist (and I still love football). At that time (I was about 12 years old) science for me was not biology, but astronomy. Biology interested me, but I remember that it seemed too 'earthly'. I was fascinated by the stars and planets so much that, together with my brother Emilio and some friends, we founded a small Astroamateurs Club (today one of the largest in Italy). Indeed, my first approach to professional science was at the age of 16, when our group had the chance on a daily basis to use one of the telescopes of the Astronomical Observatory of Naples. As an amateur, I studied astronomy for more than 10 years, and I am still a member of the Italian Astronomical Society.

When the time came to decide which direction to take after high school, I picked biology. This decision was the result of two powerful factors: my fascination for the molecular aspects of biology had increased dramatically (how could I resist magic words such as 'DNA'!), and my profound allergy to mathematics.

In 1976 I started to work for my thesis at the International Institute of Genetics and Biophysics, CNR, at the time directed by Lucio Luzzatto. My excitement was great and Watson and Crick were my heros. How was I to know that 10 years later I would have my lunch everyday at the same cafeteria as Francis Crick, at the Salk Institute! During my thesis I developed a strong interest in gene expression. The split structure of eukaryotic genes was just beginning to be uncovered and questions were starting to be raised on how the transcriptional regulation of these genes could be achieved (Ziff and Evans, 1978; Breathnach and Chambon, 1981).

With the help of one of the greatest cell biologists of our time, the late Jean Brachet, I was accepted as a post-doc in Pierre Chambon's group in Strasbourg in December 1979. This was an incredible intellectual jump for me. Pierre's team was working on several subjects, all interconnected, and all of wide biological importance. In addition, it was without doubt one of the most successful laboratories of the time and one of the first where the new techniques of molecular genetics were being applied. Pierre's group had successfully purified and characterized the eukaryotic RNA polymerases (Chambon, 1975), had uncovered the organization of chromatin (Chambon, 1978), had discovered splicing in several chicken genes, and was ready to tackle the problem of how the transcription of eukaryotic genes was regulated (Gannon *et al.*, 1979; Benoist *et al.*, 1980).

Transcription in a test tube

The major problem at that time was to develop a system that would allow correct transcription initiation by RNA polymerase II *in vitro*. With this in hand, it would be possible to transcribe a specific gene *in vitro*, and so eventually to study its promoter and the proteins required for an efficient and specific transcription. Work being carried out in several laboratories, notably those of Bob Roeder, Jim Manley and Phil Sharp, was pioneering the use of cell-free extracts (Weil *et al.*, 1979; Manley *et al.*, 1980). The system was quickly set up in Pierre's lab, and soon we were able to transcribe various genes efficiently and specifically in a test tube. Several colleagues contributed to this project. I particularly enjoyed working with Jeff Corden on the construction and analysis of the first mutants that would demonstrate the function of the TATA box (Corden *et al.*, 1980; Sassone-Corsi *et al.*, 1981). The major late promoter of adenovirus-2 deleted at position -32 was transcribed *in vitro* with the same efficiency as the wild-type promoter; however, the deletion of only three additional nucleotides downstream of position -29 had a dramatic negative effect (Corden *et al.*, 1980). Using the first directed mutagenesis techniques, gene cloning and transcriptional analysis, gave me experience for the first time of the 'new genetics' approach to studying biological problems. We could 'play around' with genes, and that for me was a thrilling experience.

The study of various promoters in transfected cells indicated that the TATA box was not everything. Soon it was clear that promoter regions upstream from the TATA box were also important for efficient transcription. Experiments from various labs showed that these upstream regions were actually different from one promoter to another, but fell into distinct groups (Grosschedl and Birnstiel, 1980; Dierks *et al.*, 1981; McKnight *et al.*, 1981; Mellon *et al.*, 1981; Grosveld *et al.*, 1982). Thus, *in vivo* studies revealed that our *in vitro* transcription systems could not faithfully reproduce all of the regulatory properties of specific promoters. Things started to change, however, when we showed that the adenovirus-2 major late promoter's upstream region, which was required for efficient expression *in vivo*, also influenced the efficiency of *in vitro* transcription (Hen *et al.*, 1982). Subsequent studies on other viral and cellular promoters confirmed this finding. These included another adenovirus promoter, E3, which I studied together with Todd Leff (Leff *et al.*, 1985). The road was now open for the identification and purification of transcription factors. Few at that time anticipated the bewildering complexity that we recognize today. Not many could imagine the miriad of transcription factors and their interactions, responsible for tissue- and

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cell-specific transcription, developmentally regulated expression and cell cycle-modulated gene control. In this light it is clear why the subsequent discovery of enhancers had a dramatic impact.

Enhancers

In 1981 the world of transcription was shocked by the manner in which some viral regulatory sequences could dramatically stimulate transcription (for reviews see Yaniv, 1982; Khoury and Gruss, 1983). In Pierre's lab the model system was SV40. It had been shown that this virus had a repeated 72 bp sequence in the early promoter which had the property of enhancing expression from both the homologous and heterologous promoters, irrespective of its orientation and even at a distance of several kilobases (Benoist and Chambon, 1981; Mathis and Chambon, 1981; Moreau *et al.*, 1981). These chacteristics did not fit with the current ideas at that time: the concept that a short piece of DNA could have such a strong intrinsic power was unexpected. These sequences, the enhancers, were then encountered in several other viral systems, and were always associated with very early viral functions (Khoury and Gruss, 1983). This priviliged position within the viral genome suggested that enhancers may be required to ensure a powerful and rapid initiation of infection. Since I was involved in work with adenovirus promoters, we started to investigate the immediate early gene E1A. A powerful and structurally complex enhancer was discovered at the extreme left-end of the viral genome (Hearing and Shenk, 1983; Sassone-Corsi *et al.*, 1983).

In 1983 I attended a small Cold Spring Harbor meeting on 'Enhancers'. It was clear that while finding enhancers was not much of a surprise anymore, the problem was to understand how they function. I was intrigued by the surprising characteristics of these sequences and I decided to look for possible *trans*-acting factors which could be involved in their function. To do so, one approach was to apply the same *in vitro* transcription system first developed to study upstream sequences. After several attempts, I succeeded in showing a clear effect of the SV40 enhancer *in vitro* (Sassone-Corsi *et al.*, 1984). The effect was specific as demonstrated by mutations in the sequence which also abolished the effect *in vivo*. More importantly, by using competition assays coupled to *in vitro* transcription, I was able to demonstrate that enhancers were indeed binding *trans*-acting factors (Sassone-Corsi *et al.*, 1985a). These experiments also demonstrated the existence of various enhancer factors, which were host-cell encoded. This notion was also confirmed by *in vivo* competition experiments (Scholer and Gruss, 1984). The importance of cell-specific factors was clearly demonstrated by the discovery of the first enhancer in a cellular gene, the heavy-chain immunoglobulin enhancer (Banerji *et al.*, 1983; Chandler *et al.*, 1983; Gillies *et al.*, 1983). Subsequent work involving extended mutagenesis of enhancer sequences (Weiher *et al.*, 1983; Zenke *et al.*, 1986) would ultimately lead to the present day explanation of their structure−function relationship.

Viral models to understand transcriptional regulation

In the meantime, in Pierre's lab, Emiliana Borrelli, Todd Leff, Colin Goding and others, were involved in the characterization of E1A adenovirus gene products. Adenovirus had been a model system for several years in Pierre Chambon's lab. Studies by Tom Shenk, Joe Nevins, Arnie Berk and others were indicating the importance of E1A products in the normal infectious cycle of the virus (Berk and Sharp, 1978; Jones and Shenk, 1979; Nevins, 1982). We established that E1A proteins acted as *trans*-activators of the expression of other early adenoviral genes (Leff *et al.*, 1984). These results were corroborated by other groups. Since that time, I have remained interested in E1A, and subsequent experiments allowed me to explore further the function of this fascinating gene. E1A appeared to activate some cellular genes (Kao and Nevins, 1983; Stein and Ziff, 1984), and I extended this observation to genes of particular interest, such as the proto-oncogenes c-*fos* and c-*myc*. Of course, the possibility for a viral *trans*-activator to induce the expression of cellular oncogenes was likely to be physiologically important (Sassone-Corsi and Borrelli, 1987). More recently, we have shown that E1A is also able to activate the proto-oncogene c-*jun* (de Groot *et al.*, 1991). These observations were particularly striking in the light of results showing that the E1A products also have a powerful transforming activity and that they can complement the oncogene *ras*, by replacing c-*myc* (Ruley, 1983).

The E1A picture was to become even more interesting by an unexpected discovery (Borrelli *et al.*, 1984). Experiments by Emiliana Borrelli, together with René Hen, revealed that E1A could also act as a transcriptional repressor of both viral and cellular enhancers (Borrelli *et al.*, 1984, 1986; Hen *et al.*, 1986). This finding was then logically linked to the observation that some viruses, such as SV40, polyoma and Moloney sarcoma virus, could not propagate in undifferentiated embryonal carcinoma (EC) cells (Swartzendruber *et al.*, 1977; Katinka *et al.*, 1981; Fujimura and Linney, 1982). The block in the normal infectious cycle of these viruses was known to be transcriptional and linked to the undifferentiated state of the cells. EC cells can be induced to differentiate *in vitro* into endodermal cells by treatment with retinoic acid (Strickland and Madhavi, 1978). Differentiated EC cells then lose their ability to restrict viral infection. In the case of polyoma, some mutant viruses that could infect EC cells had been isolated (Katinka *et al.*, 1981). The DNA sequence changes in the genome of these mutants clustered in the enhancer region, strongly suggesting that the transcriptional block at the early phases of the infection involved some repression of the enhancer function. Then, an obvious, and at the same time intriguing question was: did undifferentiated EC cells contain an E1A-like cellular function which was required to protect themselves from viral infection? This possibility was strongly supported by an important observation by Imperiale *et al.* (1984). Mutant adenoviruses lacking the E1A region, and which displayed a delayed infection cycle (E1A is required for the rapid activation of all the other early viral genes), would grow with a normal kinetic in undifferentiated EC cells. This meant that a cellular function could substitute for the absent E1A. The demonstration that there is an E1A-like activity in EC cells came from competition experiments in transfected cells. I had previously shown that the activity of various enhancers could be monitored in transiently transfected EC cells, and that this activity

changed depending on whether the cells were differentiated or not (Sassone-Corsi *et al.*, 1985b). By transfecting increasing amounts of plasmids containing exclusively enhancer sequences, I could observe the transcriptional activation of an enhancer-dependent reporter cotransfected in the same cells (Sassone-Corsi *et al.*, 1987). This effect was obtained via the titration of the negative cellular factors by the competitor.

These studies were suggestive of complex interplays among nuclear factors. The stage was set for the purification and characterization of the *trans*-acting factors responsible for the functioning of this remarkable transcriptional machinery (Serfling *et al.*, 1985; Sassone-Corsi and Borrelli, 1986).

My time in Pierre's lab was valuable. I learned molecular biology (with all the necessary tricks) and the ability to do experiments with scientific rigor and logic. In addition, I was lucky to be there at the time when the first foundations in the understanding of eukaryotic gene expression were layed. Finally, the great atmosphere of those days is still a wonderful memory, which stays alive through the tight bonds of friendship established with many of the lab fellows.

Fos in California

One of the fascinating aspects of understanding gene expression is the possibility of establishing links with physiological cellular responses, which lead to regulated proliferation and/or differentiation. That the products of several cellular oncogenes are localized in the nucleus, and more importantly that some have apparent DNA binding properties (Bishop, 1983), provided the first clues that a link might exist between the oncogenic properties of these proteins and their putative regulatory function. However, when in 1985 I decided to apply my background in gene expression to the study of nuclear oncogenes, I had no idea that I was going to experience some very exciting scientific moments. At the beginning of 1986 I moved to the Salk Institute, in San Diego. The move from central Europe to Southern California had several attractions. Scientifically, I had been following the elegant work by Inder Verma on the oncogene c-*fos*, which appeared to be a paradigm of the nuclear oncogene class (Verma, 1986). Furthermore, the Salk Institute, together with other prestigious institutions in San Diego such as the Scripps and the University of California, constitutes a remarkable place to do research. I enjoyed my time in San Diego, where I had the opportunity to interact with a large number of excellent scientists. Inder's lab, in addition, was a great place to work, where a nice group of people succeeded in mixing intense research work with a friendly atmosphere. I also had the chance of interacting with other scientists at Salk, such as Ron Evans and his team.

Soon after my arrival in Inder's lab I realized that I had been right in judging the tremendous potential of the c-*fos* gene, as transforming product and potential transcriptional regulator. Early work had shown that the oncogene *fos* is the resident transforming gene of both the FBJ-murine osteosarcoma virus (FBJ-MSV) and FBR-MSV. The FBJ-MSV was isolated from a spontaneous bone tumor in a CF1 mouse, whereas FBR-MSV was isolated from a radiation-induced bone tumor (Verma and Graham, 1987). Both viruses, however, cause transformation of fibroblasts *in vitro* and induce osteosarcomas *in vivo*. Over 90% of mice infected with the *fos* viruses develop tumors associated with bone. These tumors often arise on several bones and sometimes in the peritoneum. This indicates multiple sites of viral tumor formation, although metastases are not seen (Verma and Graham, 1987). A 55 kDa phosphoprotein encoded by the oncogene (v-*fos*) was identified by immunoprecipitation using sera from rats that had been injected with FBJ-MSV-transformed cells (Curran and Teich, 1982; Curran *et al.*, 1982). Such rats developed tumors. In addition their sera precipitated a 39 kDa protein of host origin (Curran and Teich, 1982; Curran *et al.*, 1984). A few years later the p39 protein was to dominate the attention of many researchers. We and others would show that this Fos-associated protein is structurally and functionally related to the product of another nuclear oncogene, c-*jun* (see next section; Chiu *et al.*, 1988; Rauscher *et al.*, 1988; Sassone-Corsi *et al.*, 1988c).

Early response genes

Oncogenes can be classified functionally into three broad categories: (i) growth factors and receptors; (ii) mediators of intracellular signal transduction pathways; and (iii) regulators of gene expression (Bishop, 1985). It seemed likely that interaction and cooperation among the products of the different classes of proto-oncogene might dramatically influence cell growth, differentiation and development. Thus, it was becoming clear that oncogenes would not only be responsible for a transformed phenotype, but that they could play an important role in normal cell physiology. This concept was justified by the notion that several oncogenes had been found to encode proteins with important functions. For example, the oncogene v-*sis* product bears homology to a subunit of the secreted platelet-derived growth factor and the *erb*B and *fms* oncogene products have striking homologies to growth factor receptors (Doolittle *et al.*, 1983; Waterfield *et al.*, 1983; Sherr *et al.*, 1985).

When I started to analyze the characteristics of c-*fos*, I realized that it shared many common features with other nuclear oncogenes: (i) a rapid and often transient induction in response to numerous agents capable of promoting either growth and development, or inducing differentiation; (ii) a short half-life of messenger RNA, which may in part be due to the presence of adenine-thymine (AT)-rich destabilizing sequences in the 3′ untranslated region; (iii) the Fos protein also has a short half-life of 20–90 min; and (iv) the nuclear oncoproteins are invariably post-translationally modified, usually by serine phosphorylation. Overall, nuclear oncoproteins appeared to have a deliberate transient function presumably because their sustained expression could have the potential to induce cellular transformation (Verma and Sassone-Corsi, 1987).

The inducible nature of c-*fos* was intriguing. Early work had shown that genes responding to growth factor stimulation of resting cells can be divided into temporal classes. Those activated prior to the onset of S phase are referred to as 'early' genes, and those responding thereafter are called 'late' genes, in analogy with viral genes expressed sequentially during virus development (Lau and Nathans, 1985). The first set of early genes to respond

('immediate-early' genes) appeared to be transcriptionally activated generally within minutes of serum stimulation and their expression did not require new protein synthesis (Cochran *et al.*, 1983). In fact, they are superinduced by growth factor in the presence of an inhibitor of protein synthesis, due both to prolonged transcription and to stabilization of their normally labile mRNAs (Greenberg and Ziff, 1984; Lau and Nathans, 1987). Since activation of immediate-early genes did not require new protein synthesis, it seemed likely that it must be triggered by the modification of pre-existing transcription factor(s). A second set of early genes ('delayed-early' genes) was shown to be activated within hours of serum or PDGF stimulation (Linzer and Nathans, 1983), and unlike immediate-early genes, it was shown that their expression is blocked by protein synthesis inhibitors. Their activation was therefore thought to depend on synthesis of immediate-early proteins. Within this scenario, it appeared that *fos* may play a crucial role in the putative regulation of downstream, delayed-early genes. Thus, two problems had to be tackled: (i) how the *fos* gene could be activated so rapidly and what determined its transcriptional attenuation, and (ii) what the Fos protein does. I decided to work on both points, thinking that the two could be linked.

A paradigm of inducible transcription (and of autoregulation)

A hallmark of the c-*fos* gene is its inducibility by a variety of agents including mitogens, hormones, ionophores, differentiation-specific agents, stress, drugs, etc. Induction is invariably very rapid and transient (Greenberg and Ziff, 1984; Kruijer *et al.*, 1984). The analysis of the c-*fos* promoter was started by Richard Treisman, whose pioneering work allowed the identification of the region required for serum stimulation (Treisman, 1985). A number of laboratories followed Richard's steps, so that today the *fos* regulatory sequences have been extensively analyzed. An inducible element, termed SRE (serum responsive element), is in the upstream region and encompasses a sequence with dyad symmetry, between −297 and −317. The SRE is required for induction of the c-*fos* gene by serum growth factors, phorbol esters, nerve growth factor and Ras-induced signalling (Fisch *et al.*, 1987; Gilman, 1988; Visvader *et al.*, 1988; Sassone-Corsi *et al.*, 1989). The SRE binding protein itself, SRF, was subsequently purified from HeLa cell nuclear extracts by affinity chromatography (Treisman, 1986, 1987). SRF, whose molecular mass is 67 kDa, binds to the SRE as a dimer. More recently it has been shown that SRF binding activity is regulated by physical interaction with other proteins (Janknecht *et al.*, 1993; Marais *et al.*, 1993). However, it is still not clear how this complex activates transcription in response to serum and growth factors. It is evident, however, that post-translational modifications such as phosphorylation, modulate the ability of the complex to interact with other components of the transcriptional machinery.

Experiments on the SRE explained the inducibility of the c-*fos* gene by serum growth factors and phorbol esters. In addition, c-*fos* expression was also known to be stimulated by activators of the adenylyl cyclase pathway or by calcium; however, an SRE-containing reporter was shown to be uninducible by these agents. I decided to look into the possibility that the *fos* promoter may have a modular structure, where various sites might be targets of differential signalling pathways. I identified a site, at position −60, which displayed some homology to the somatostatin gene cAMP-responsive element (CRE, a palindrome TGACGTCA). By using transient transfection assays, footprinting experiments and other approaches, I demonstrated that the −60 element indeed acts as a CRE (Sassone-Corsi *et al.*, 1988a). This result was then confirmed by other groups, which also showed that the same site would respond to changes in calcium concentration (Berkowitz *et al.*, 1989; Fisch *et al.*, 1989).

Transcriptional stimulation of c-*fos* involved factors existing in the cell prior to induction. Treatment with cycloheximide not only allowed induction, but elicited a prolonged and more powerful expression (Kruijer *et al.*, 1984). This notion told us something important about the transcriptional attenuation, known to follow the induction and characteristic of the transient activation of early response genes. I was intrigued by the attenuation mechanism and I wondered whether the *fos* gene might have all the properties to ensure its own negative autoregulation. In a previous study I demonstrated the existence of negative regulation of the c-*fos* promoter (Sassone-Corsi and Verma, 1987). Then, in a series of experiments we demonstrated that this was indeed the case. The *de novo* synthesized Fos protein was shown to block the promoter activity by altering the structure of the transcriptional complex interacting with the SRE (Sassone-Corsi *et al.*, 1988b). This negative feedback mechanism was the first to be described for a proto-oncogene; its significance was evident: it allowed the fine tuning of intracellular Fos protein levels, and thus might function as a cyclic control of cellular proliferation. Results from other laboratories confirmed our obsevations (Shaw *et al.*, 1989; Lucibello *et al.*, 1990). Another exciting facet of those experiments was given by the observation that the Fos protein appeared to be associated with p39, the oncogenic product of c-*jun* (Sassone-Corsi *et al.*, 1988c).

The Fos–Jun association

Early work by Peter Vogt allowed the cloning of the v-*jun* oncogene from avian sarcoma virus 17 (ASV17) isolated from a spontaneous sarcoma in an adult chicken (Vogt and Bos, 1989). ASV17 was also shown to induce oncogenic transformation in chick embryo fibroblast (CEF) cultures. *In vitro*-transformed cells have fusiform shape, growing in a tightly packed parallel array on solid substrates under agar, and are able to form anchorage-independent colonies in semi-solid medium. Chick embryo fibroblasts transformed by ASV17 *in vitro* are not immortal, regardless of whether they produce virus or not. In contrast, cultures derived from ASV17-induced tumors have a much longer lifespan, suggesting that they have undergone additional changes that are not seen in cell culture (Vogt and Bos, 1989).

The ASV17 transformation-specific protein $p65^{gag-jun}$ is a fusion protein. Immunofluorescence studies revealed that the v-*jun* protein is localized in the nucleus of CEF transfected with ASV17 (Bos *et al.*, 1988). A crucial step in the understanding of Jun function was made when nucleotide sequence analysis indicated that the C-terminus of v-*jun* is similar to the C-terminus of the yeast transcrip-

tional activator GCN4 (Vogt *et al.*, 1987), which regulates the expression of genes involved in amino acid biosynthesis. The conserved region is restricted to the C-terminal portion of v-*jun*, which has 44% homology with the DNA binding domain of GCN4. Thus it was proposed that v-*jun* might encode a sequence-specific DNA binding protein (Vogt *et al.*, 1987). An important clue that v-*jun* might have a normal cellular counterpart encoding a sequence-specific DNA binding factor came with the discovery that the core consensus DNA sequence, ATGACTCAT, recognized by GCN4, is very similar to the binding site of the human *trans*-activator protein AP-1 (Bohmann *et al.*, 1987; Lee *et al.*, 1987). Indeed, the cloning of the cellular counterpart of v-*jun* paved the way for a set of crucial experiments, required for the understanding of how cooperativity between two transcription factors in gene regulation may be correlated with cooperativity between oncogenes. The c-*jun* gene was shown to be an early response gene induced by mitogenic stimuli (Lamph *et al.*, 1988; Quantin and Breathnach, 1988; Ryseck *et al.*, 1988). More importantly, I demonstrated that the c-Jun protein associated with c-Fos to stimulate binding to an AP-1 site (also called TRE, TPA-responsive element) (Sassone-Corsi *et al.*, 1988c). Stimulation in binding correlated with an enhanced *trans*-activation of a TRE-containing reporter (Sassone-Corsi *et al.*, 1988c). Similar data were obtained in other laboratories (Chiu *et al.*, 1988). I was greatly excited by these results which indicated the crucial role of the Fos–Jun association in the regulation of gene expression and, more importantly, the link with the transformed phenotype.

The leucine zipper

How did Fos and Jun interact? Did they associate as dimers, or in another way? If they formed dimers, which combinations would determine AP-1 activity? Which protein domain is required for the association? Very soon it became clear that the AP-1 binding activity is composed of the products of various genes, following the discovery of several other *fos* and *jun* family members (as example see Ryder *et al.*, 1989). This observation was an indication of the high versatility and complexity of the system.

In order to understand the mechanisms regulating Fos–Jun association, I started a series of experiments using truncated proteins and specific antibodies. It became clear that the structure required for the association was the so-called leucine zipper (Sassone-Corsi *et al.*, 1988d). This structure was originally described by Landschulz and colleagues for the mammalian transcription factor CCAAT/enhancer binding protein (C/EBP) (Landschulz *et al.*, 1988). Alignment of the primary structure with that of Fos, Jun and the yeast regulatory protein GCN4 revealed a common region of homology consisting of a heptad repeat of leucine residues. Central to the model which was subsequently developed to explain the structure of this region was the notion that all these factors bind to DNA as dimeric complexes (Busch and Sassone-Corsi, 1990). My experiments, together with others from various research groups, indicated that altering the organization of the leucine residues in the repeats dramatically affected association and DNA binding of the dimer (Kouzarides and Ziff, 1988; Sassone-Corsi *et al.*, 1988d). While the leucine zipper was demonstrated to be required exclusively for the dimerization function, adjacent basic-rich domains were shown to be involved in direct DNA contact (Gentz *et al.*, 1989; Schuermann *et al.*, 1989; Turner and Tjian, 1989). The basic domain–leucine zipper structure was defined bZip, and became the hallmark of a large family of transcription factors. Finally, it was demonstrated that Fos and Jun proteins function as dimers and dimerization brings the two basic domains, located adjacent to the leucine zippers, together to form a bimolecular DNA binding domain (Gentz *et al.*, 1989). Members of the Fos family, which do not homodimerize, are consequently unable to bind to DNA on their own. In contrast, Jun proteins can bind DNA either as homodimers or as heterodimeric complexes with Fos proteins.

The heptad leucine repeat region was predicted to form an amphipathic α-helix with the leucine residues aligned along one ridge (Landschulz *et al.*, 1988). Furthermore, by modelling it was demonstrated that two of these helices could associate in a coiled-coil conformation. This central feature was then confirmed by a series of studies investigating the structural properties of GCN4 leucine zipper peptides (O'shea *et al.*, 1989). The overall structure of the bZip domain has been predicted by two similar models; termed the 'scissors-grip' and 'induced helical fork' models (Vinson *et al.*, 1989). In both, the repeating leucines lie at the interface between two parallel helices and thus are reminiscent of the teeth of a metallic zipper. Immediately N-terminal to the leucine zipper, lies a 30 amino acid conserved region, rich in basic amino acids. The predicted α-helical conformation of the leucine zipper extends through the basic region and thus the coiled-coil region of the dimer juxtaposes the paired basic regions to form a Y-shaped structure where the arms are the basic regions and the stem is formed by the coiled coil (Vinson *et al.*, 1989). In this structure the α-helical basic region is able to wrap around the DNA helix, contacting bases in the major groove. The 'scissors-grip' and 'induced helical fork' models differ in the details of the basic domain–DNA interactions. Consistent with these models is that the spacing between the basic region and the leucine zipper is a highly conserved feature, and thus the orientation of basic region helices relative to the leucine zipper helices appears critical.

Factors modulating Fos–Jun function

In 1989 I left San Diego and returned to Strasbourg. I decided to study the role of ancillary factors that may modulate Fos–Jun function. Alterations in AP-1 function can be brought about by transcriptional induction or by post-translational modifications of both oncoproteins and their regulatory factors. These modifications, which take place in the absence of protein synthesis, and the ancillary factors which are involved in modulating AP-1 function, can act at two levels: DNA binding and transcriptional activation.

Experiments by various groups have indicated that Fos–Jun function can be modulated by several elements. Post-translational modifications have an important role: both Fos and Jun have been found to be phosphorylated and modulated by various kinases (Barber and Verma, 1987; Tratner *et al.*, 1990; Angel and Karin, 1991; Binétruy *et al.*, 1991; de Groot and Sassone-Corsi, 1992; Derijard *et al.*, 1994); in addition the DNA binding function of the

Fos–Jun dimer appears to be regulated by reduction–oxidation (Xanthoudakis *et al.*, 1992). However, DNA binding can also be altered by interaction of AP-1 with other proteins. Cross-family dimerization between bZip transcription factors of the Fos–Jun and ATF groups can also alter DNA binding specificity. For instance, heterodimerization of c-Jun with ATF-2 changes its specificity of binding from an AP-1 site to a cAMP-responsive element (Ivashkiv *et al.*, 1989). Alternatively, AP-1 function can be blocked by repressor factors to which it does not dimerize (Sassone-Corsi *et al.*, 1990); in particular, this is the case for CREM (Masquilier and Sassone-Corsi, 1992), which is able to bind to an AP-1 site and thus block Jun-mediated *trans*-activation via the occupation of the regulatory site. These are examples of cross-talk mechanisms at the nuclear level, which may integrate information derived from multiple upstream signal transduction pathways.

Another demonstration of cross-talk and of a factor which influences AP-1 function was described in my lab by Johan Auwerx. The protein, termed IP-1, is present both in the cytoplasm and nucleus of cells and reduces AP-1 complex formation with DNA in a rapid and phosphorylation-dependent fashion (Auwerx and Sassone-Corsi, 1991). The IP-1 protein appears to be very unstable. IP-1 is regulated by phosphorylation and only in its non-phosphorylated form exerts an inhibitory activity on AP-1 DNA binding. IP-1 itself is the subject of complex regulation. Specifically, IP-1 activity was shown to be modulated after activation of several signal transduction pathways, including PKC, PKA and Ca^{2+}/calmodulin-dependent kinase pathways, as well as following serum stimulation of cells (Auwerx and Sassone-Corsi, 1992). Additional examples of proteins which may influence Fos–Jun function have also been described by other groups (Baichwal and Tjian, 1990; Bengal *et al.*, 1992; Oehler and Angel, 1992). Amongst these, of particular importance are the steroid/retinoic acid receptors (as examples see Gaub *et al.*, 1990; Schüle *et al.*, 1990). Thus, results from several groups have shown that there are many potential mechanisms whereby factors which influence cell growth and differentiation might modulate the interplay between Fos–Jun and nuclear receptors to bring about a dynamic pleiotropic response.

In conclusion, it is clear that AP-1 is able to interact with a multitude of additional regulatory proteins. How these cofactors are able to modulate AP-1 activity and its phosphorylation state is still unclear. Additional studies will be required to fully unravel the molecular architecture and the physiological functions of the AP-1 complex.

The nuclear response to cAMP

The links between signal transduction and gene expression were clearly established from the example of Fos–Jun. Other cases, such as SRF and NF-κB, demonstrated that many transcription factors constituted important nuclear targets of intracellular second messengers. Increase in the intracellular levels of cyclic 3′–5′ adenosine monophosphate (cAMP) is generated by the activation of the membrane associated enzyme adenylyl cyclase. This activation occurs upon stimulation of specific receptors by their ligands, via coupling by GTP binding proteins (Borrelli *et al.*, 1992; Lalli and Sassone-Corsi, 1994). cAMP, in turn, binds cooperatively to the two binding sites of the regulatory subunit of protein kinase A (PKA), releasing the catalytic subunit from constitutive inhibition. PKA is then translocated from its cytoplasmic and Golgi complex anchoring sites and actively phosphorylates its substrate (the serine in the context X-Arg-Arg-X-Ser-X). This target sequence is encountered in a number of both cytoplasmic and nuclear proteins (Nigg *et al.*, 1985).

It is remarkable that the elevation of intracellular cAMP levels can result in either stimulation or repression of specific gene expression, suggesting that complex, cell-specific molecular mechanisms must operate in the nucleus. Activated PKA appears to modulate the function of nuclear factors that bind to DNA sequences present in the promoter regions of cAMP-inducible genes (Habener, 1990; Lalli and Sassone-Corsi, 1994). Most of these genes, which are often expressed in neuroendocrine cells, contain one or a few cAMP-response elements (CREs) (Comb *et al.*, 1986; Sassone-Corsi, 1988; Borrelli *et al.*, 1992). CREs are constituted by the palindromic sequence TGACGTCA, or variations of it, which is strikingly similar to the binding site of transcription factor AP-1 (Borrelli *et al.*, 1992). This notion further supports the existence of nuclear cross-talk mechanisms which I have mentioned before (Sassone-Corsi *et al.*, 1990; Masquilier and Sassone-Corsi, 1992).

Transcription à la CREM

One of my preoccupations was to link the molecular mechanisms of gene expression and the physiology of neuroendocrine systems. The first nuclear factor to be cloned which is able to bind a CRE site was CREB (Hoeffler *et al.*, 1988). Subsequently, CREB has been followed by many other proteins which all belong to the bZip transcription factor class (for review see Lalli and Sassone-Corsi, 1994). The CRE binding factors are highly homologous in their bZip region, while they diverge in other parts of the protein. While heterodimerization between different factors is possible, there appears to be a specific 'dimerization code', that allows only some specific combinations to be made (Hai *et al.*, 1989; Ivashkiv *et al.*, 1989).

In 1990, Nick Foulkes in my lab cloned the CREM gene. The discovery of this gene opened a new dimension in the study of the transcriptional response to cAMP (Foulkes *et al.*, 1991a). This is due to the remarkable dynamic and modular genomic structure of the gene, which also offers clues to the understanding of the generation of functional diversity in transcription factors generally. CREM is the first gene known to encode multiple CRE binding proteins with either antagonistic or activator function.

The CREM gene was isolated in collaboration with Emiliana Borrelli by screening a cDNA library from mouse pituitary at low stringency with oligonucleotides corresponding to the leucine zipper and basic region of CREB. The logic behind this approach is that the adenylyl cyclase pathway plays an important role in the modulation of the hormonal regulation in the pituitary gland. At that time we were amazed by a striking feature of the CREM cDNA: the presence of two DNA binding domains. The first is complete and contains a leucine zipper and basic region very similar to CREB; the second is located in the

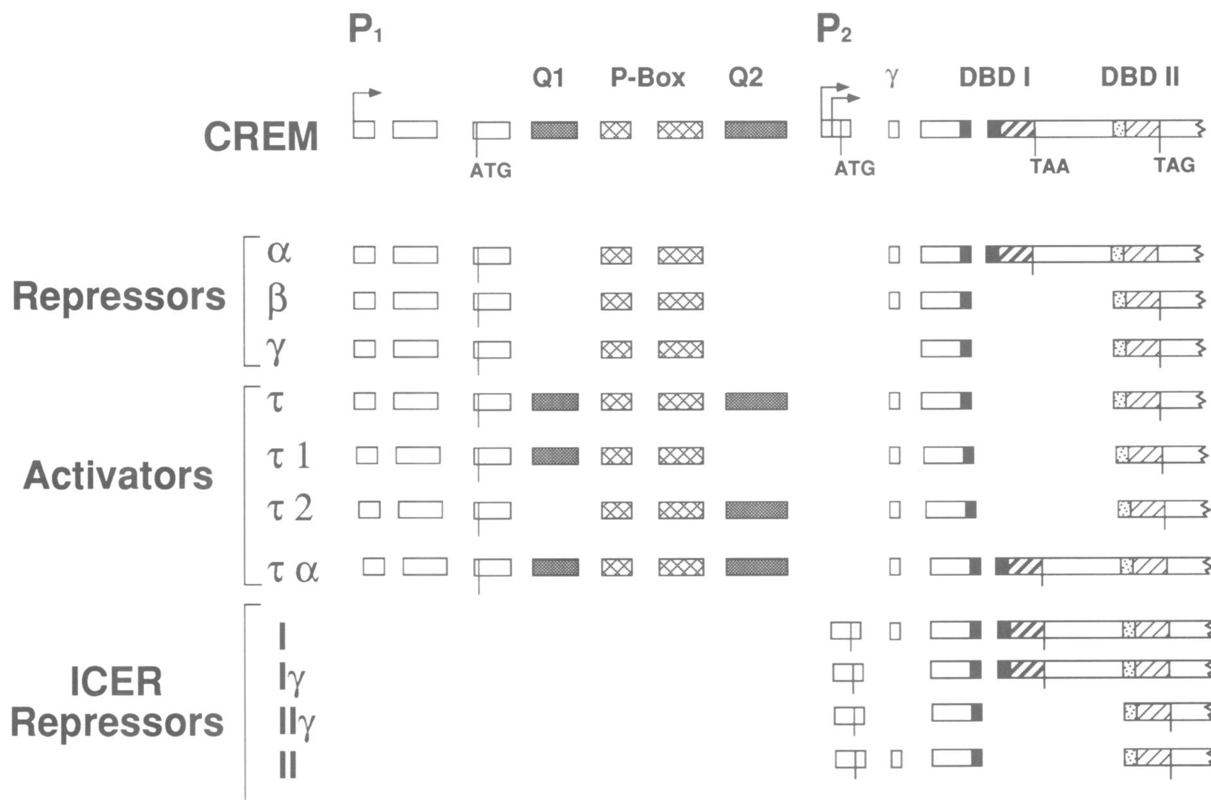

Fig. 1. Activators and repressors from the same gene. Top: schematic representation of the CREM gene. Exons encoding the glutamine-rich domains (Q1 and Q2), the P-box, the γ domain (γ) and the two alternative DNA binding domains (DBDI and DBDII) are shown. Below are represented the various activator and repressor isoforms which have been described to date. The P1 promoter is GC-rich and directs a non-inducible pattern of expression. Also represented is the ICER family. All the ICER transcripts are derived from an internal start-site of transcription (P2) located between the Q2 and γ-exon. A family of four types of ICER transcript is generated by alternative splicing of the DBDs and γ-domain exons; ICER-I, ICER-Iγ, ICER-II and ICER-IIγ.

3′ untranslated region of the gene, out of phase with the main coding region, and contains a half basic region and a leucine zipper more divergent from CREB. Since that time, various mRNA isoforms have been identified that are obtained by differential cell-specific splicing (Laoide *et al.*, 1993). Alternative usage of the two DNA binding domains was demonstrated in various tissues and cell types, where quite different patterns of expression were found (Foulkes *et al.*, 1991a; Mellstrom *et al.*, 1993). This strongly contrasts with the expression of CREB and ATFs which are generally ubiquitous (Hai *et al.*, 1989; Habener, 1990) and are considered to function as constitutive regulators.

Since the beginning of our study, CREM expression appeared to be finely regulated, both transcriptionally and post-transcriptionally. In fact, not only cell- and tissue-specific expression was observed, but also the production of isoforms with different function. Three products with antagonistic activity were the first to be described (Foulkes *et al.*, 1991a). These isoforms revealed alternative usage of the two DNA binding domains (α and β isoforms, see Figure 1), as well as a small deletion of 12 amino acids (γ isoform). The potential for even more complexity of CREM regulation is hinted at by its usage of alternative poly(A) addition sites. This combined with the presence or absence of 10 AUUUA sequences in the 3′ untranslated region may modulate mRNA instability (Shaw and Kamen, 1986). The strict cell- and tissue-specific expression of CREM is indicative of a pivotal function in the regulation of cell-specific cAMP responses. This suggests that CREM might occupy a central control point in the pituitary gland, since it is known that the physiology of this gland is finely regulated by a multiplicity of hormones whose signal transduction pathways involve adenylyl cyclase. Interestingly, other well described examples of cell-specific splicing include the genes encoding neuronal peptides and hormones in brain and pituitary cells (Leff *et al.*, 1986). It thus appears clear that cell-specific splicing is a crucial mechanism of CREM regulation, which modulates the DNA binding specificity as well as the activity of the final CREM products (see Figure 1).

Later we found that CREM is particularly remarkable since it can encode both activators and repressors of CRE-mediated transcription (Delmas *et al.*, 1992; Foulkes and Sassone-Corsi, 1992). This property is unique to CREM within this family of transcription factors. The first CREM activator that we encountered was CREMτ (Foulkes *et al.*, 1992; Laoide *et al.*, 1993). This is generated by alternative splicing which incorporates two additional exons encoding glutamine-rich domains.

The key role of phosphorylation in transcriptional activation

The transcriptional activation domain of both CREB and CREMτ contains two independent regions. The first, indicated as phosphorylation box (P-box) or kinase-

inducible domain (KID), contains several phosphorylation sites for various kinases (see Figure 1; de Groot *et al.*, 1993a). The second region, divided into two parts, flanks the P-box at the N- and C-termini, and is glutamine rich (Q-rich domain) (Gonzalez *et al.*, 1991; Foulkes *et al.*, 1992). Upon activation of the adenylyl cyclase pathway, a serine residue at position 133 of CREB and at position 117 of CREMτ is phosphorylated by PKA (Gonzalez *et al.*, 1991; de Groot *et al.*, 1993a); this phosphorylation event appears to be required for activation of transcription. This interpretation of the functional role of PKA-dependent phosphorylation in the activation phenomenon is now judged simplistic. Work on the CREM activator protein has shown that other kinases participate in the transcriptional activation. The same serine 117 in CREMτ has been shown to be the target for phosphorylation for PKC, calmodulin kinase and $p34^{cdc2}$ *in vitro* (de Groot *et al.*, 1993a,b); *in vivo* treatment with forskolin, serum, TPA or Ca^{2+} ionophore A23187 all lead to enhanced phosphorylation of Ser117 (de Groot *et al.*, 1993a, 1994). Thus, a single phosphorylation site appears to be the target for the action of different signal transduction pathways, resulting in a complex response at the level of CRE-regulated gene expression. Moreover, cooperativity of phosphorylation by casein kinases I and II and by GSK-3 and PKA has been shown within the P-box of CREMτ (de Groot *et al.*, 1993a).

The two regions flanking the P-box of CREB and CREM are particularly glutamine rich, and function as transcriptional activation domains when tested in the GAL4 assay (Laoide *et al.*, 1993), similarly to domains present in other activators, such as AP-2 and Sp1. They can function as activators also when present singly, as in the CREM isoforms τ1 and τ2, which contain, respectively, only the first (Q_1) or the second (Q_2) glutamine-rich domain of CREMτ. Their action is additive on the magnitude of transcriptional stimulation, with Q_2 being more powerful than Q_1 (Laoide *et al.*, 1993).

What is the mechanism by which the interaction between a phosphorylated P-box and one or two glutamine-rich domains leads to transcriptional activation? The current notion is that phosphorylation causes a change in the conformational structure of the activator protein, exposing the glutamine-rich domains to interact with the components of the basal transcriptional machinery (Gonzalez *et al.*, 1991). The P-box is also able to confer PKA inducibility on a heterologous acidic activation domain, *in trans* as well as *in cis* , so it could be involved also in the regulation of the transcriptional activation domains present in other factors bound on nearby sites on the promoter (Brindle *et al.*, 1993). Verification of this model awaits determination of the crystal structure of unphosphorylated and phosphorylated CREB and/or CREMτ. The effect of phosphorylation on DNA binding by CREB and CREMτ is less clear and remains controversial.

Transcriptional repression and the role of ICER

Dephosphorylation seems to be an important mechanism in the negative regulation of CREB activity; after the initial phosphorylation by PKA, CREB is dephosphorylated *in vivo* by the protein phosphatase PP-1, leading to transcriptional attenuation of the c-*fos* gene (Hagiwara *et al.*, 1992). Moreover, both PP-1 and PP-2A can dephosphorylate CREB *in vitro*, resulting in an apparent decreased binding to low affinity CRE sites (Hagiwara *et al.*, 1992). Negative regulation, however, also seems to be brought about by CRE binding repressor factors.

The CREM gene encodes most of the known repressors of CRE-induced transcription. Their characteristic tissue-specific distribution is suggestive of key physiological roles for these proteins. Two groups of CRE element binding repressors can be distinguished:
(i) constitutively expressed repressors, whose activity can be modulated by phosphorylation; and
(ii) repressors whose synthesis is stimulated by cAMP.

The first CREM repressors to be characterized, CREMα, β and γ, belong to the first group (Foulkes *et al.*, 1991a,b; Laoide *et al.*, 1993). Their structure is similar to the activator CREMτ; they all contain the P-box, but lack both the glutamine-rich domains Q_1 and Q_2 (Laoide *et al.*, 1993); this allows them to bind to CRE elements, but they are unable to stimulate the basal transcription machinery, thus behaving functionally as repressors. We have also shown that their repressor activity is partially diminished by phosphorylation *in vivo* with PKA (Laoide *et al.*, 1993). Thus, induction of cAMP-responsive transcription via PKA function is likely to be determined by the combined activation of the activators and down-regulation of CREM antagonists.

A new insight into the molecular mechanisms underlying the physiological cAMP-dependent repression of gene expression is given by the recent discovery in the lab of a new family of CREM isoforms, ICER (Inducible cAMP Early Repressor), whose expression is inducible by cAMP (see Figure 1; Molina *et al.*, 1993). An alternative, intronic promoter (P2; see Figure 1) within the CREM gene directs transcription of short transcripts which encode the smallest CRE binding nuclear factors described to date (Stehle *et al.*, 1993). These can contain either the first or the second DNA binding domain and can include or exclude the γ-domain, but they lack the P-box and both the Q-rich domains. The ICER proteins are the most efficient repressors of CRE-mediated transcription. The characteristic kinetics of ICER expression show that CREM belongs to the class of the immediate 'early response' genes. Indeed, there is a rapid increase in CREM transcript after cAMP stimulus, with a peak at 2–4 h, and a rapid down-regulation, delayed by cycloheximide treatment (Molina *et al.*, 1993).

The remarkably small size of the ICER proteins makes them some of the smallest known transcription factors. It is interesting to note that a protein that consists essentially of the dimerization region/DNA binding domain acts as the most powerful repressor of CRE-induced transcription (Molina *et al.*, 1993). This is in agreement with deletion studies that show that the bZip domain is sufficient for full antagonism (Granger-Schnarr *et al.*, 1992; Laoide *et al.*, 1993). These notions suggest that repressors function by occupying the CRE sites as inactive homodimers or that they dimerize with activators, blocking them by the formation of non-functional heterodimers.

The ICER promoter (P2) is strongly cAMP inducible. cAMP inducibility is conferred by a 181 bp upstream region (Molina *et al.*, 1993; E.Lalli, in preparation). A cluster of four CRE sites in this CREM promoter is responsible for the inducibility. Importantly, since CREM

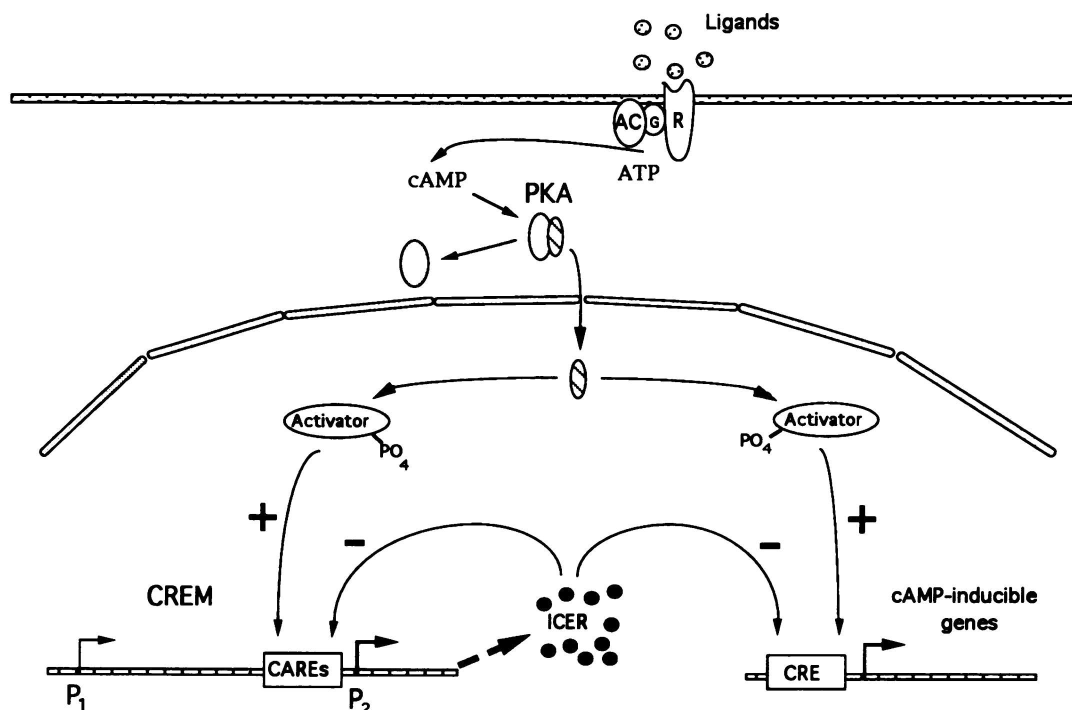

Fig. 2. The role of ICER in the regulation of gene expression by cAMP. Schematic representation of the cAMP signal transduction pathway operating from the cell membrane, through the cytoplasm and into the nucleus. Ligands interacting with transmembrane receptors (R) stimulate the enzyme adenylyl cyclase (AC) via interactions with G-proteins (G). The subsequent rise in intracellular cAMP concentration results in the dissociation of the regulatory and catalytic subunits of PKA and the translocation of active catalytic subunits into the nucleus. PKA phosphorylates and thereby stimulates transcriptional activators binding to CREs (activators, e.g. CREB and CREMτ) which induce transcription from the promoters of cAMP-responsive genes. These factors activate transcription from the CREM P_2 promoter via the CRE elements and ultimately lead to a rapid increase in ICER protein levels. ICER represses cAMP-induced transcription, including that from its own promoter. The consequent fall in ICER protein levels eventually leads to a release of repression and permits a new cycle of transcriptional activation.

inducibility is enhanced and prolonged by cycloheximide treatment of cAMP-stimulated cells, it is likely that a *de novo* synthesized protein could be responsible for its transient nature (see Figure 2). Indeed, the decline of CREM expression after induction is due to the newly synthesized ICER proteins which bind to the ICER promoter, permitting negative autoregulation of transcription. So ICER represents the first repressor described whose function is regulated by cAMP primarily by modulation of its intracellular levels and not by phosphorylation. The production of an inducible repressor could represent, according to the cell type and the physiological situation, a mechanism for the attenuation of gene expression after the first burst of activation by cAMP (Figure 2).

Physiological importance of CREM

The crucial role of cAMP-dependent signalling implies that CRE binding factors are likely to play a role *in vivo* mainly in neuroendocrine processes. The CREB family of proteins has been shown to play a fundamental role in the ontogeny of the pituitary somatotroph cells (Struthers *et al.*, 1991). A transgenic mouse strain expressing a CREB protein mutated in the PKA phosphoacceptor site under the growth hormone promoter control was generated. The phenotype of the mice was dwarf, with atrophic pituitary and an absence of somatotroph cells, reminiscent of the cellular knock-out transgenics where the somatotroph lineage was ablated (Borrelli *et al.*, 1989). Interestingly, however, animals whose CREB gene has been inactivated by homologous recombination are not dwarf (Hummler *et al.*, 1994), suggesting some functional redundancy of bZip factors in pituitary development.

In the lab we have obtained results demonstrating that CREM plays an important role in spermatogenesis, where there exists a striking differential regulation of CREM expression according to the developmental stage (see Figure 3; Foulkes *et al.*, 1992). Premeiotic germ cells express only the repressor isoforms at low levels, while, from the pachytene spermatocyte stage onward, the activator CREMτ is expressed at very high levels (see Figure 3; Delmas *et al.*, 1993). Removal of the pituitary causes reappearance of the preswitch pattern; the change in CREM isoform pattern is regulated by the pituitary hormone FSH and does not occur at the transcriptional level. It was established that stabilization of the CREM transcript is mediated by differential usage of polyadenylation sites in the 3′ untranslated region, eliciting the exclusion of most of the destabilizer elements present 3′ of the stop codon in post-meiotic cells (Foulkes *et al.*, 1993). Moreover, candidate target genes for CREMτ in the germ cells have been identified, among which is RT7, a gene highly expressed in spermatids (Delmas *et al.*, 1993).

Very recently CRE binding factors have also been shown to be involved in circadian rhythms. In the hypothalamic suprachiasmatic nucleus, which bears the endogenous biological clock, CREB is phosphorylated on Ser133 after a light stimulus (Ginty *et al.*, 1993). We have shown that CREM expression is strikingly regulated in the rat pineal gland with a circadian rhythm, with maximal levels present

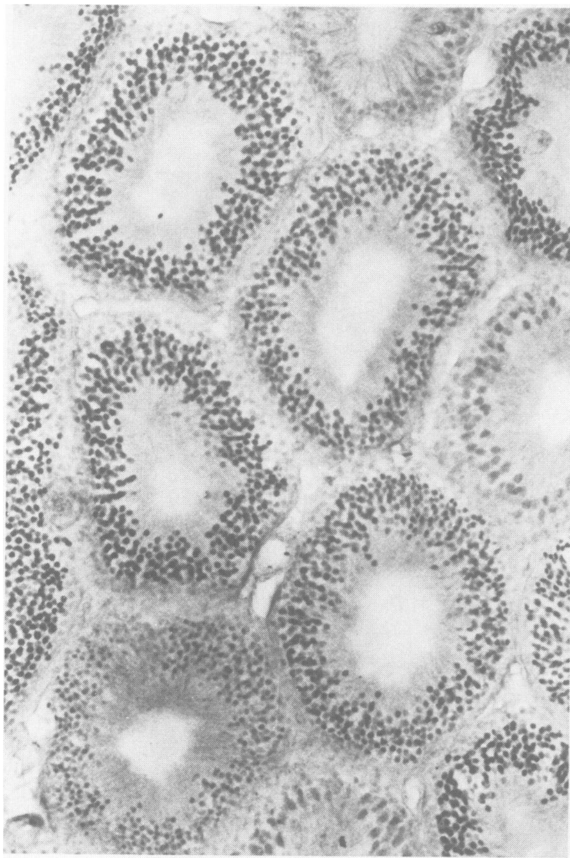

Fig. 3. CREMτ is expressed stage specifically in developing spermatids. Peroxidase staining of rat seminiferous tubules showing expression of the CREM protein in spermatids. The CREM antibody used for this experiment was prepared against a bacterially produced CREMτ protein. Note the differential intensity of staining in the various tubules, indicating that CREM expression is developmentally regulated.

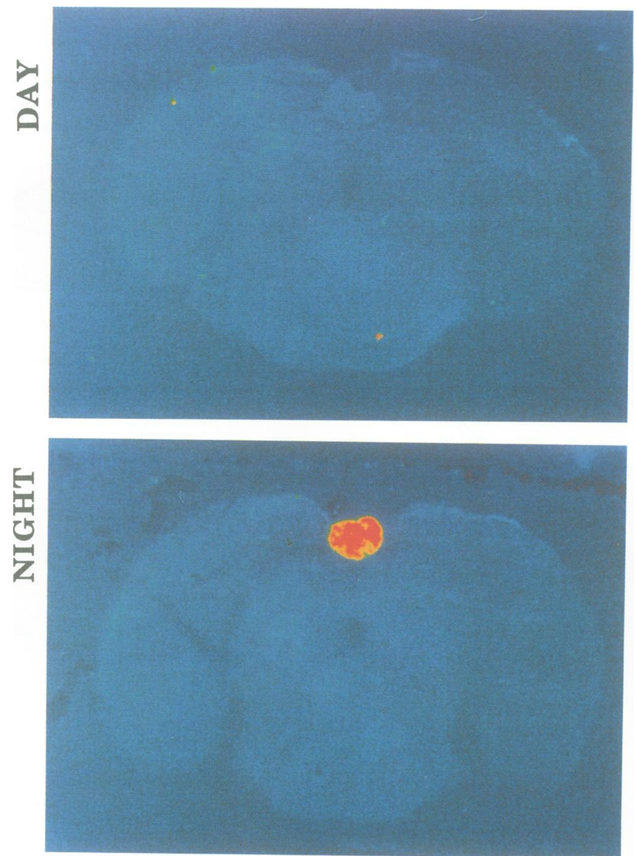

Fig. 4. CREM expression is elevated at night in the pineal gland. *In situ* hybridization of brain sections with an antisense CREM-specific riboprobe. Rats maintained in 12 h light/12 h dark conditions (light on 07:00) were sacrificed at consecutive timepoints. Representative sections from 12:00 (DAY) and 02:00 (NIGHT) are shown. An intense hybridization signal is present in the pineal gland at night corresponding to ICER transcripts induced by clock-derived adrenergic signals (this experiment was performed by J.Stehle).

at night (see Figure 4), and is elicited by adrenergic stimulation of the cAMP pathway (Stehle *et al.*, 1993). Interestingly, the CREM products generated in a circadian fashion correspond to ICER, whose promoter is cyclically activated by adrenergic signals sent by the suprachiasmatic nucleus. The functional implications of generating oscillating levels of a powerful repressor in the pineal gland are of central physiological interest. Studies are in progress to establish whether CREM cyclic expression could be involved in the rhythmic synthesis of the pineal hormone melatonin.

Further clues about the physiological role played by the CREM proteins will be given by gene knockout by homologous recombination. However, the functional redundancy present in this transcription factor family, which could substitute for the lack of function of a specific gene, should be considered. Interestingly, the knockout of the CREB gene causes no apparent phenotype, but in the homozygote −/− animals CREM gene transcripts are increased ~3-fold (Hummler *et al.*, 1994). However, the unique inducibility feature of the CREM gene, together with its clear involvement in neuroendocrine processes, suggest that the *in vivo* deletion of the gene is likely to have important consequences.

Conclusion

A prerequisite for normal cell growth and differentiation is that each cell must be able to receive, interpret and respond appropriately to signals from other cells and the environment. The plasma membrane is the external interface of the cell and bears many elements which are required for the primary analysis of such signals. Binding of a ligand to its receptor initiates a cascade of events which modulate a variety of cellular functions, including the control of gene expression. By altering the spectrum of genes expressed, the cell appropriately modifies its physiology for a given stimulus. Unfortunately, aberrations in this process can occur and may lead to deregulated cell proliferation and ultimately tumorigenesis. Thus, our improved understanding of gene expression is also of great utility. I was fortunate to be involved in these studies. If I could start all over again, I would make the same choices (and I would probably still need to give up football!).

Acknowledgements

The description of the events reported in this article is a personal account and thus cannot be completely objective. It is impossible to document the specific contribution of all the individuals participating in scientific

progress in such a field. Many which could not be cited in the text will find themselves in the references. To those colleagues and friends that I have missed and who feel that they should have been mentioned, I apologize. I thank Pierre Chambon and Inder M.Verma for their crucial contribution to my formation as a scientist. I am grateful to all the friends and collaborators who have helped me in these years. I personally wish to mention Jeff Corden, Giovanna Camerino, Todd Leff, Colin Goding and John Sisson, and also A.Giangrande, J.Atwater, V.Raymond, J.Visvader, W.Lamph, C.L.Li, P.Augereau, H.Boeuf, C.Hauss, M.Acker, R.Elkaim, L.Maroteaux, R.Hen, B.Boulay, I.Tratner, J.Naranjo and all the members of Chambon's and Verma's labs during my stay. Since I started my research group in Strasbourg many have contributed to the team work. I am especially thankful to Nicholas Foulkes and Florence Schlotter, who were there from the beginning. I also thank the past and present members of the lab, which is currently composed of Enzo Lalli, Carlos Molina, Janet Lee, Lucia Monaco, François Nantel and Barbara Bardoni. I am grateful to Emiliana Borrelli who has helped me throughout these years; her advice and consideration have determined many of the events described in this article. Finally I like to thank Lucio and Emilio. I dedicate my work and this award to my parents, Mario and Anna, who programmed me their way.

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